

Claims

What is claimed is:

- 5        1.        A cigarette comprising:  
              an increased-nicotine transgenic plant or plant portion of a species of the genus  
*Nicotiana*, wherein said plant or plant portion expresses at least one heterologous  
nucleic acid that up-regulates the production of nicotine in such transgenic plant or  
plant portion; and
- 10                a tar-to-nicotine yield ratio of between about 3 and about 8, as measured by the  
FTC or ISO method.
- 15        2.        The cigarette according to claim 1, wherein said increased-nicotine transgenic  
plant or plant portion exhibits increased nicotine as compared to a non-transformed  
parent plant or plant portion from which said transgenic plant or plant portion is  
produced.
- 20        3.        The cigarette according to claim 1, wherein said increased-nicotine transgenic  
plant or plant portion, as compared to a non-transformed control plant or plant  
portion, contains and expresses a heterologous DNA encoding at least a segment of an  
enzyme required for the biosynthesis of nicotine in tobacco, said transgenic plant or  
plant portion exhibiting increased levels of said enzyme as compared to a non-  
transformed control plant or plant portion and increased-nicotine content as compared  
to a non-transformed control plant or plant portion.
- 25        4.        The cigarette according to claim 3, wherein said enzyme is selected from a  
group consisting of arginine decarboxylase (ADC), methylputrescine oxidase (MPO),  
NADH dehydrogenase, ornithine decarboxylase (ODC), phosphoribosylanthranilate  
isomerase (PRAI), putrescine N-methyltransferase (PMT), quinolate phosphoribosyl  
transferase (QPT), and S-adenosyl-methionine synthetase (SAMS).
- 30        5.        The cigarette according to claim 1, wherein said plant species is *Nicotiana  
tabacum*.

6. The cigarette according to claim 5, wherein said yield is about 1 mg of tar and between about 0.12 mg and about 0.34 mg of nicotine.

7. The cigarette according to claim 5, wherein said yield is about 2 mg of tar and between about 0.25 mg and about 0.68 mg of nicotine.

8. The cigarette according to claim 5, wherein said yield is about 3 mg of tar and between about 0.36 mg and about 1.0 mg of nicotine.

9. The cigarette according to claim 5, wherein said yield is about 4 mg of tar and between about 0.50 mg and about 1.36 mg of nicotine.

10. The cigarette according to claim 5, wherein said yield is about 5 mg of tar and between about 0.62 mg and about 1.70 mg of nicotine.

11. The cigarette according to claim 5, wherein said yield is about 6 mg of tar and between about 0.75 mg and about 2.0 mg of nicotine.

12. The cigarette according to claim 5, wherein said yield is about 7 mg of tar and between about 0.87 mg and about 2.33 mg of nicotine.

13. The cigarette according to claim 5, wherein said yield is about 8 mg of tar and between about 1.0 mg and about 2.66 mg of nicotine.

14. The cigarette according to claim 1, wherein said yield ratio is greater than about 3 and less than about 5.

15. A cigarette comprising an increased-nicotine transgenic plant or plant portion of a species of the genus *Nicotiana*, wherein said plant or plant portion expresses at least one heterologous nucleic acid that up-regulates the production of nicotine in said transgenic plant or plant portion.

16. The cigarette according to claim 15, wherein said increased-nicotine transgenic plant or plant portion exhibits increased nicotine as compared to a non-transformed parent plant or plant portion from which said transgenic plant or plant portion is produced.

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17. The cigarette according to claim 15, wherein said increased-nicotine transgenic plant or plant portion, as compared to a non-transformed control plant or plant portion, contains and expresses a heterologous DNA encoding at least a segment of an enzyme required for the biosynthesis of nicotine in tobacco, said transgenic plant exhibiting increased levels of said enzyme as compared to a non-transformed control plant or plant portion and increased-nicotine content as compared to a non-transformed control plant or plant portion.

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18. The cigarette according to claim 17, wherein said enzyme is selected from the group consisting of arginine decarboxylase (ADC), methylputrescine oxidase (MPO), NADH dehydrogenase, ornithine decarboxylase (ODC), phosphoribosylanthranilate isomerase (PRAI), putrescine N-methyltransferase (PMT), quinolate phosphoribosyl transferase (QPT), and S-adenosyl-methionine synthetase (SAMS).

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19. The cigarette according to claim 15, wherein said plant species is *Nicotiana tabacum*.

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20. A method of making a cigarette comprising:

providing an increased-alkaloid transgenic plant or plant portion, as compared to a non-transformed control plant or plant portion, of a species of the genus *Nicotiana*;

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crossing said plant with a plant of the species *Nicotiana tabacum* to produce a progeny plant, wherein said progeny plant has an increased-nicotine phenotype; and

producing a cigarette comprising said progeny plant and having a tar-to-nicotine yield ratio of between about 3 and about 8, as measured by the FTC or ISO method.

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21. The cigarette of claim 20.

22. The cigarette according to claim 21, wherein said transgenic plant species is *Nicotiana tabacum*.

23. A method of making a cigarette comprising:

5 providing a reduced-nicotine transgenic plant or plant portion, as compared to a non-transformed control plant or plant portion, of the species *Nicotiana tabacum*;

crossing said reduced-nicotine plant with a plant of the species *Nicotiana rustica* to obtain a progeny transgenic plant or plant portion; and

producing a cigarette comprising said progeny plant or plant portion.

10 24. The method according to claim 23, wherein said progeny plant or plant portion contains increased nicotine as compared to said reduced-nicotine plant or plant portion.

15 25. The method according to claim 23, wherein said progeny plant or plant portion contains decreased nicotine as compared to said plant species *Nicotiana rustica*.

20 26. The cigarette of claim 23.

27. The cigarette according to claim 26 and having a tar-to-nicotine yield ratio of between about 3 and about 8, as measured by the FTC or ISO method.

28. A method of making a cigarette comprising:

25 providing an increased-nicotine transgenic plant or plant portion, as compared to a non-transformed control plant or plant portion, of a species of the genus *Nicotiana*, wherein said plant or plant portion expresses at least one heterologous nucleic acid that up-regulates the production of nicotine in said transgenic plant or plant portion;

30 producing reconstituted tobacco from said plant or plant portion; and  
producing a cigarette comprising said reconstituted tobacco.

29. The cigarette of claim 28.

30. The cigarette according to claim 29, wherein said increased-nicotine transgenic plant or plant portion exhibits increased nicotine as compared to a non-transformed parent plant or plant portion from which said transgenic plant or plant portion is produced.

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31. The cigarette according to claim 29, wherein said increased-nicotine transgenic plant or plant portion, as compared to a non-transformed control plant or plant portion, contains and expresses a heterologous DNA encoding at least a segment of an enzyme required for the biosynthesis of nicotine in tobacco, said transgenic plant or  
10 plant portion exhibiting increased levels of said enzyme as compared to a non-transformed control plant or plant portion and increased-nicotine content as compared to a non-transformed control plant or plant portion.

32. The cigarette according to claim 31, wherein said enzyme is selected from the  
15 group consisting of arginine decarboxylase (ADC), methylputrescine oxidase (MPO), NADH dehydrogenase, ornithine decarboxylase (ODC), phosphoribosylanthranilate isomerase (PRAI), putrescine N-methyltransferase (PMT), quinolate phosphoribosyl transferase (QPT), and S-adenosyl-methionine synthetase (SAMS).

33. The cigarette according to claim 29 and having a tar-to-nicotine yield ratio of between about 3 and about 8, as measured by the FTC or ISO method

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34. A method of making a cigarette comprising:

providing an increased-nicotine transgenic plant or plant portion, as compared  
25 to a non-transformed control plant or plant portion, of a species of the genus *Nicotiana*, wherein said plant or plant portion expresses at least one heterologous nucleic acid that up-regulates the production of nicotine in said transgenic plant or plant portion;

producing expanded tobacco from said plant or plant portion; and

30 producing a cigarette comprising said expanded tobacco.

35. The cigarette of claim 34.

36. The cigarette according to claim 35, wherein said increased-nicotine transgenic plant or plant portion exhibits increased nicotine as compared to a non-transformed parent plant or plant portion from which said transgenic plant or plant portion is produced.

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37. The cigarette according to claim 35, wherein said increased-nicotine transgenic plant or plant portion, as compared to a non-transformed control plant or plant portion, contains and expresses a heterologous DNA encoding at least a segment of an enzyme required for the biosynthesis of nicotine in tobacco, said transgenic plant or  
10 plant portion exhibiting increased levels of said enzyme as compared to a non-transformed control plant or plant portion and increased-nicotine content as compared to a non-transformed control plant or plant portion.

38. The cigarette according to claim 37, wherein said enzyme is selected from the  
15 group consisting of arginine decarboxylase (ADC), methylputrescine oxidase (MPO), NADH dehydrogenase, ornithine decarboxylase (ODC), phosphoribosylanthranilate isomerase (PRAI), putrescine N-methyltransferase (PMT), quinolate phosphoribosyl transferase (QPT), and S-adenosyl-methionine synthetase (SAMS).

20 39. A cigarette comprising:

a transgenic plant or plant portion of a species of the genus *Nicotiana* that exhibits increased nicotine as compared to a non-transformed parent plant or plant portion from which said transgenic plant or plant portion is produced, wherein said plant or plant portion expresses at least one heterologous nucleic acid that up-  
25 regulates the production of nicotine in said transgenic plant or plant portion; and

a lower tar-to-nicotine yield ratio as compared to a control cigarette comprising said non-transformed parent plant or plant portion.

40. The cigarette according to claim 39, wherein said increased-nicotine transgenic  
30 plant or plant portion, as compared to said non-transformed control plant or plant portion, contains and expresses a heterologous DNA encoding at least a segment of an enzyme required for the biosynthesis of nicotine in tobacco, said transgenic plant or plant portion exhibiting increased levels of said enzyme as compared to said non-

transformed control plant and increased-nicotine content as compared to said non-transformed control plant or plant portion.

41. The cigarette according to claim 40, wherein said enzyme is selected from the group consisting of arginine decarboxylase (ADC), methylputrescine oxidase (MPO), NADH dehydrogenase, ornithine decarboxylase (ODC), phosphoribosylanthranilate isomerase (PRAI), putrescine N-methyltransferase (PMT), quinolate phosphoribosyl transferase (QPT), and S-adenosyl-methionine synthetase (SAMS).

42. The cigarette according to claim 39, wherein said plant species is *Nicotiana tabacum*.

43. The cigarette of claim 39, wherein said yield ratio is greater than about 3 and less than about 5.

44. A method of making a cigarette comprising:

providing an increased-nicotine transgenic plant or plant portion, as compared to a non-transformed control plant or plant portion, of a species of the genus *Nicotiana*, wherein said plant or plant portion expresses at least one heterologous nucleic acid that up-regulates the production of nicotine in said transgenic plant or plant portion;

extracting nicotine from said transgenic plant or plant portion;

providing a plant or plant portion of a species of the genus *Nicotiana*;

adding said extracted nicotine to said species to form increased nicotine plant material;

producing a cigarette comprising said increased nicotine plant material.

45. The method according to claim 44, wherein said nicotine is nicotine salts of organic acids.

46. The cigarette of claim 44.

47. The cigarette according to claim 46, wherein said increased-nicotine transgenic plant or plant portion exhibits increased nicotine as compared to a non-transformed parent plant or plant portion from which said transgenic plant or plant portion is produced.

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48. The cigarette according to claim 46, wherein said increased-nicotine transgenic plant or plant portion, as compared to a non-transformed control plant or plant portion, contains and expresses a heterologous DNA encoding at least a segment of an enzyme required for the biosynthesis of nicotine in tobacco, said transgenic plant or  
10 plant portion exhibiting increased levels of said enzyme as compared to a non-transformed control plant or plant portion and increased-nicotine content as compared to a non-transformed control plant or plant portion.

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49. The cigarette according to claim 48, wherein said enzyme is selected from the group consisting of arginine decarboxylase (ADC), methylputrescine oxidase (MPO), NADH dehydrogenase, ornithine decarboxylase (ODC), phosphoribosylanthranilate isomerase (PRAI), putrescine N-methyltransferase (PMT), quinolate phosphoribosyl transferase (QPT), and S-adenosyl-methionine synthetase (SAMS).

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50. The cigarette according to claim 46, wherein said plant species is *Nicotiana tabacum*.

51. The cigarette of claim 46 and having a tar-to-nicotine yield ratio of between about 3 and about 8, as measured by the FTC or ISO method.

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52. The cigarette according to claim 51, wherein said yield is about 1 mg of tar and between about 0.12 mg and about 0.34 mg of nicotine.

53. The cigarette according to claim 51, wherein said yield is about 2 mg of tar  
30 and between about 0.25 mg and about 0.68 mg of nicotine.

54. The cigarette according to claim 51, wherein said yield is about 3 mg of tar and between about 0.36 mg and about 1.0 mg of nicotine.



55. The cigarette according to claim 51, wherein said yield is about 4 mg of tar and between about 0.50 mg and about 1.36 mg of nicotine.

56. The cigarette according to claim 51, wherein said yield is about 5 mg of tar and between about 0.62 mg and about 1.70 mg of nicotine.

57. The cigarette according to claim 51, wherein said yield is about 6 mg of tar and between about 0.75 mg and about 2.0 mg of nicotine.

58. The cigarette according to claim 51, wherein said yield is about 7 mg of tar and between about 0.87 mg and about 2.33 mg of nicotine.

59. The cigarette according to claim 51, wherein said yield is about 8 mg of tar and between about 1.0 mg and about 2.66 mg of nicotine.

60. The cigarette according to claim 51, wherein said yield ratio is greater than about 3 and less than about 5.

61. A method of making a cigarette comprising:

providing an increased-nicotine transgenic plant or plant portion, as compared to a non-transformed control plant or plant portion, of a species of the genus *Nicotiana*, wherein said plant or plant portion expresses at least one heterologous nucleic acid that up-regulates the production of nicotine in said transgenic plant or plant portion;

extracting nicotine from said transgenic plant or plant portion;  
providing an second plant or plant portion of a species of the genus *Nicotiana*;  
adding said extracted nicotine to said second plant or plant portion to form increased nicotine plant material; and

producing a cigarette comprising said increased nicotine plant material and having a lower tar-to-nicotine yield ratio as compared to a control cigarette comprising said second plant or plant portion without the addition of said extracted nicotine.

62. The method according to claim 61, wherein said nicotine is nicotine salts of organic acids.

63. The cigarette of claim 61.

64. The cigarette according to claim 63, wherein said increased-nicotine transgenic plant or plant portion exhibits increased nicotine as compared to a non-transformed parent plant or plant portion from which said transgenic plant or plant portion is produced.

65. The cigarette according to claim 63, wherein said increased-nicotine transgenic plant or plant portion, as compared to a non-transformed control plant or plant portion, contains and expresses a heterologous DNA encoding at least a segment of an enzyme required for the biosynthesis of nicotine in tobacco, said transgenic plant or plant portion exhibiting increased levels of said enzyme as compared to a non-transformed control plant or plant portion and increased-nicotine content as compared to a non-transformed control plant or plant portion.

66. The cigarette according to claim 65, wherein said enzyme is selected from the group consisting of arginine decarboxylase (ADC), methylputrescine oxidase (MPO), NADH dehydrogenase, ornithine decarboxylase (ODC), phosphoribosylanthranilate isomerase (PRAI), putrescine N-methyltransferase (PMT), quinolate phosphoribosyl transferase (QPT), and S-adenosyl-methionine synthetase (SAMS).

67. The cigarette according to claim 63, wherein said plant species is *Nicotiana tabacum*.

68. A method of making a cigarette comprising:

providing a reduced-nicotine transgenic plant or plant portion, as compared to a non-transformed control plant or plant portion, of a species of the genus *Nicotiana*, wherein said plant or plant portion expresses at least one heterologous nucleic acid that down-regulates the production of nicotine in said transgenic plant or plant portion;

producing cigarette tobacco from said transgenic plant or plant portion;  
adding nicotine to said cigarette tobacco; and  
producing a cigarette comprising said cigarette tobacco and having a tobacco-specific nitrosamines level below about 0.5 micrograms per gram of tobacco.

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69. The method according to claim 68, wherein said nicotine is nicotine salts of organic acids, nicotine analogs or synthesized nicotine.

70. The cigarette of claim 68.

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71. The cigarette according to claim 70, wherein said reduced-nicotine transgenic plant or plant portion exhibits reduced nicotine as compared to the parent plant or plant portion from which said transgenic plant or plant portion is produced.

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72. The cigarette according to claims 70, wherein said reduced-nicotine transgenic plant or plant portion contains and expresses a heterologous DNA encoding at least a segment of an enzyme required for the biosynthesis of nicotine in tobacco, said transgenic plant or plant portion exhibiting reduced levels of said enzyme as compared to a non-transformed control plant or plant portion and reduced-nicotine content as compared to a non-transformed control plant or plant portion.

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73. The cigarette according to claim 72, wherein said enzyme is selected from the group consisting of arginine decarboxylase (ADC), methylputrescine oxidase (MPO), NADH dehydrogenase, ornithine decarboxylase (ODC), phosphoribosylanthranilate isomerase (PRAI), putrescine N-methyltransferase (PMT), quinolate phosphoribosyl transferase (QPT), and S-adenosyl-methionine synthetase (SAMS).

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74. The cigarette according to claim 70, wherein said plant species is *Nicotiana tabacum*.

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75. The cigarette according to claim 70, wherein said tobacco-specific nitrosamines level is less than about 0.05 micrograms (50ppb) per gram of tobacco.

76. The cigarette according to claim 70 and having a tar-to-nicotine yield ratio of between about 3 and about 8.

77. A method of making a tobacco product comprising:

- 5 providing a transgenic plant or plant portion of a species of the genus *Nicotiana* that exhibits reduced nicotine as compared to a non-transformed parent plant or plant portion from which said transgenic plant or plant portion is produced, wherein said plant or plant portion expresses at least one heterologous nucleic acid that down-regulates the production of nicotine in said transgenic plant or plant
- 10 portion;
- producing tobacco from said transgenic plant or plant portion;
- adding nicotine to said tobacco; and
- producing a product comprising said tobacco and having a lower tobacco-specific nitrosamines level as compared to a control product comprising said non-
- 15 transformed parent plant or plant portion.

78. The method according to claim 77, wherein said nicotine is nicotine salts of organic acids or synthesized nicotine.

20 79. The tobacco product of claim 77.

80. The tobacco product according to claim 79, wherein said reduced-nicotine transgenic plant or plant portion contains and expresses a heterologous DNA encoding at least a segment of an enzyme required for the biosynthesis of nicotine in tobacco,

25 said transgenic plant or plant portion exhibiting reduced levels of said enzyme as compared to a non-transformed control plant or plant portion and reduced-nicotine content as compared to a non-transformed control plant or plant portion.

81. The tobacco product according to claim 80, wherein said enzyme is selected

30 from the group consisting of arginine decarboxylase (ADC), methylputrescine oxidase (MPO), NADH dehydrogenase, ornithine decarboxylase (ODC), phosphoribosylanthranilate isomerase (PRAI), putrescine N-methyltransferase (PMT),

quinolate phosphoribosyl transferase (QPT), and S-adenosyl-methionine synthetase (SAMS).

82. The tobacco product according to claim 79, wherein said plant species is  
5 *Nicotiana tabacum*.

83. A tobacco product according to claim 79, wherein said tobacco-specific nitrosamines level is below about 1 microgram per gram (1 ppm).

10 84. The tobacco product according to claim 79, wherein said tobacco product is in a form selected from a group consisting of leaf tobacco, shredded tobacco and cut tobacco.

15 85. The tobacco product according to claim 79, wherein said tobacco product is selected from a group consisting of snuff, pipe tobacco, cigar tobacco, chewing tobacco and cigarette tobacco.

86. A method of making expanded tobacco comprising:  
providing an increased-nicotine transgenic plant or plant portion, as compared  
20 to a non-transformed control plant or plant portion, of a species of the genus *Nicotiana*, wherein said plant or plant portion expresses at least one heterologous nucleic acid that up-regulates the production of nicotine in said transgenic plant or plant portion;  
producing tobacco from said transgenic plant or plant portion; and  
25 expanding said tobacco.

87. The expanded tobacco of claim 86.

30 88. The expanded tobacco of claim 87, wherein said increased-nicotine transgenic plant or plant portion exhibits increased nicotine as compared to a non-transformed parent plant or plant portion from which said transgenic plant or plant portion is produced.

89. The expanded tobacco according to claim 87, wherein said increased-nicotine transgenic plant or plant portion, as compared to a non-transformed control plant or plant portion, contains and expresses a heterologous DNA encoding at least a segment of an enzyme required for the biosynthesis of nicotine in tobacco, said transgenic plant or plant portion exhibiting increased levels of said enzyme as compared to a non-transformed control plant or plant portion and increased-nicotine content as compared to a non-transformed control plant or plant portion.

90. The expanded tobacco according to claim 89, wherein said enzyme is selected from the group consisting of arginine decarboxylase (ADC), methylputrescine oxidase (MPO), NADH dehydrogenase, ornithine decarboxylase (ODC), phosphoribosylanthranilate isomerase (PRAI), putrescine N-methyltransferase (PMT), quinolate phosphoribosyl transferase (QPT), and S-adenosyl-methionine synthetase (SAMS).

91. The expanded tobacco according to claim 87, wherein said plant species is *Nicotiana tabacum*.

92. A method of making reconstituted tobacco comprising:

providing plant material selected from a group consisting of (a) an increased-nicotine transgenic plant or plant portion, as compared to a non-transformed control plant or plant portion, of a species of the genus *Nicotiana*, wherein said plant or plant portion expresses at least one heterologous nucleic acid that up-regulates the production of nicotine in said transgenic plant or plant portion and said cigarette, (b) a reduced-nicotine transgenic plant or plant portion, as compared to a non-transformed control plant or plant portion, of a species of the genus *Nicotiana*, wherein said plant or plant portion expresses at least one heterologous nucleic acid that down-regulates the production of nicotine in said transgenic plant or plant portion, (c) deproteinized tobacco fiber, and (d) freeze-dried tobacco; and reconstituting said plant material.

93. The reconstituted tobacco of claim 92.

94. A method of making reconstituted tobacco comprising:

providing a reduced-nicotine transgenic plant or plant portion, as compared to a non-transformed control plant or plant portion, of a species of the genus *Nicotiana*, wherein said plant or plant portion expresses at least one heterologous nucleic acid that down-regulates the production of nicotine in said transgenic plant or plant portion; and

reconstituting said transgenic plant or plant portion.

95. The reconstituted tobacco of claim 94.

96. The reconstituted tobacco according to claim 95, wherein said reduced-nicotine transgenic plant or plant portion exhibits reduced nicotine as compared to the parent plant or plant portion from which said transgenic plant or plant portion is produced.

97. The reconstituted tobacco according to claims 95, wherein said reduced-nicotine transgenic plant or plant portion contains and expresses a heterologous DNA encoding at least a segment of an enzyme required for the biosynthesis of nicotine in tobacco, said transgenic plant or plant portion exhibiting reduced levels of said enzyme as compared to a non-transformed control plant or plant portion and reduced-nicotine content as compared to a non-transformed control plant or plant portion.

98. The reconstituted tobacco according to claim 97, wherein said enzyme is selected from the group consisting of arginine decarboxylase (ADC), methylputrescine oxidase (MPO), NADH dehydrogenase, ornithine decarboxylase (ODC), phosphoribosylanthranilate isomerase (PRAI), putrescine N-methyltransferase (PMT), quinolate phosphoribosyl transferase (QPT), and S-adenosyl-methionine synthetase (SAMS).

99. The reconstituted tobacco according to claim 95, wherein said plant species is selected from a group consisting of *Nicotiana tabacum* and *Nicotiana rustica*.

100. The reconstituted tobacco according to claim 95, and further comprising reconstituted deproteinized tobacco fiber.

101. A cigarette comprising the reconstituted tobacco of claim 95 and having a reduced yield of tobacco-specific nitrosamines (TSNA) as compared to a control cigarette comprising said non-transformed parent plant or plant portion.

102. The cigarette according to claim 101, wherein said TSNA is selected from a group consisting of N'-nitrosonornicotine (NNN), 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butan-one (NNK), N'-nitrosoanatabine (NAT), and N'-nitrosoanabasine (NAB).

103. The cigarette according to claim 101, wherein said cigarette has a reduced yield of compounds selected from a group consisting of benzo(a)pyrene, phenols, and catechols as compared to a control cigarette comprising said non-transformed parent plant or plant portion.

104. A method of making reconstituted tobacco comprising:  
providing an increased-nicotine transgenic plant or plant portion, as compared to a non-transformed control plant or plant portion, of a species of the genus *Nicotiana*, wherein said plant or plant portion expresses at least one heterologous nucleic acid that up-regulates the production of nicotine in said transgenic plant or plant portion; and  
reconstituting said plant or plant portion.

105. The reconstituted tobacco of claim 104.

106. The reconstituted tobacco of claim 105, wherein said increased-nicotine transgenic plant or plant portion exhibits increased nicotine as compared to a non-transformed parent plant or plant portion from which said transgenic plant or plant portion is produced.



107. The reconstituted tobacco according to claim 105, wherein said increased-nicotine transgenic plant or plant portion, as compared to a non-transformed control plant or plant portion, contains and expresses a heterologous DNA encoding at least a segment of an enzyme required for the biosynthesis of nicotine in tobacco, said transgenic plant exhibiting increased levels of said enzyme as compared to a non-transformed control plant or plant portion and increased-nicotine content as compared to a non-transformed control plant or plant portion.

108. The reconstituted tobacco according to claim 107 wherein said enzyme is selected from the group consisting of arginine decarboxylase (ADC), methylputrescine oxidase (MPO), NADH dehydrogenase, ornithine decarboxylase (ODC), phosphoribosylanthranilate isomerase (PRAI), putrescine N-methyltransferase (PMT), quinolate phosphoribosyl transferase (QPT), and S-adenosyl-methionine synthetase (SAMS).

109. The reconstituted tobacco according to claim 105, wherein said plant species is *Nicotiana tabacum*.

110. The method according to claim 104, and further comprising freeze-drying said transgenic plant or plant portion after harvesting it.

111. A tobacco variety characterized by a high-nicotine trait and a high cured reducing sugar content, wherein said trait is conferred by a transgene.

112. A tobacco variety according to claim 111, wherein said variety is true-breeding for at least one of said high-nicotine trait and said sugar content.

113. A tobacco variety according to claim 111, wherein said sugar content ranges from between about 14 percent and about 30 percent.

114. A tobacco variety characterized by a high-nicotine trait and high fatty acid content, wherein said trait is conferred by a transgene.

115. A tobacco variety according to claim 114, wherein said variety is true-breeding for at least one of said high-nicotine trait and said fatty acid content.

116. A method for increasing nicotine and cured reducing sugar content in a plant,  
5 comprising:

transforming a plant having elevated cured reducing sugar content with a transgene conferring an increased nicotine phenotype;

regenerating progeny plants from said transformed plant; and

10 selecting a progeny plant having increased nicotine and cured reducing sugar content.

117. A method for increasing nicotine in a plant, comprising:

transforming a plant having elevated fatty acid synthesis with a transgene conferring an increased nicotine phenotype;

15 regenerating progeny plants from said transferred plant; and

selecting a progeny plant having increased nicotine and fatty acid levels.

118. A tobacco variety characterized by a high-nicotine trait and a cured reducing sugar content of at least 15 percent, wherein said trait is conferred by a transgene.

119. A tobacco variety according to claim 118, wherein said variety is true-breeding for at least one of said trait and said cured reducing sugar content.

120. A tobacco variety accord to claim 111, wherein said variety has a total alkaloid  
25 content of greater than 3.5 percent.

121. A tobacco variety characterized by a high-nicotine content of greater than 3.3 percent and a high cured reducing sugar trait, wherein said trait is conferred by a transgene.

122. A tobacco variety according to claim 121, wherein said variety is true-breeding for at least one of said sugar trait and said nicotine content.

123. A tobacco variety according to claim 121, wherein said cured reducing sugar content of said variety is greater than 15 percent.

124. A cigarette comprising:

an increased-nicotine transgenic plant or plant portion of a species of the genus *Nicotiana*;

a tar-to-nicotine yield ratio of between about 3 and about 8, as measured by the FTC or ISO method; and

a filler having a sugar to nicotine ratio of greater than about 3.5.

125. The cigarette according to claim 124, wherein said increased-nicotine transgenic plant or plant portion exhibits increased nicotine as compared to a non-transformed parent plant or plant portion from which said transgenic plant or plant portion is produced.

126. The cigarette according to claim 124, wherein said increased-nicotine transgenic plant or plant portion contains and expresses a heterologous nucleic acid that up-regulates the production of nicotine in said transgenic plant or plant portion.

127. The cigarette according to claim 124, wherein said increased-nicotine transgenic plant or plant portion, as compared to a non-transformed control plant or plant portion, contains and expresses a heterologous DNA encoding at least a segment of an enzyme required for the biosynthesis of nicotine in tobacco, said transgenic plant or plant portion exhibiting increased levels of said enzyme as compared to a non-transformed control plant or plant portion and increased-nicotine content as compared to a non-transformed control plant or plant portion.

128. The cigarette according to claim 127, wherein said enzyme is selected from a group consisting of arginine decarboxylase (ADC), methylputrescine oxidase (MPO), NADH dehydrogenase, ornithine decarboxylase (ODC), phosphoribosylanthranilate isomerase (PRAI), putrescine N-methyltransferase (PMT), quinolate phosphoribosyl transferase (QPT), and S-adenosyl-methionine synthetase (SAMS).

129. A cigarette comprising:

an increased-nicotine transgenic plant or plant portion of a species of the genus *Nicotiana*;

a tar-to-nicotine yield ratio of between about 3 and about 8, as measured by the  
5 FTC or ISO method; and  
cigarette smoke having a pH of greater than about 6.

130. The cigarette according to claim 129, wherein said increased-nicotine  
transgenic plant or plant portion exhibits increased nicotine as compared to a non-  
10 transformed parent plant or plant portion from which said transgenic plant or plant  
portion is produced.

131. The cigarette according to claim 130, wherein said increased-nicotine  
transgenic plant or plant portion contains and expresses a heterologous nucleic acid  
15 that up-regulates the production of nicotine in said transgenic plant or plant portion.

132. The cigarette according to claim 129, wherein said increased-nicotine  
transgenic plant or plant portion, as compared to a non-transformed control plant or  
plant portion, contains and expresses a heterologous DNA encoding at least a segment  
20 of an enzyme required for the biosynthesis of nicotine in tobacco, said transgenic plant  
or plant portion exhibiting increased levels of said enzyme as compared to a non-  
transformed control plant or plant portion and increased-nicotine content as compared  
to a non-transformed control plant or plant portion.

133. The cigarette according to claim 132, wherein said enzyme is selected from a  
group consisting of arginine decarboxylase (ADC), methylputrescine oxidase (MPO),  
NADH dehydrogenase, ornithine decarboxylase (ODC), phosphoribosylanthranilate  
isomerase (PRAI), putrescine N-methyltransferase (PMT), quinolate phosphoribosyl  
transferase (QPT), and S-adenosyl-methionine synthetase (SAMS).

134. A cigarette comprising:

an increased-nicotine transgenic plant or plant portion of a species of the genus  
*Nicotiana*;

a tar-to-nicotine yield ratio of between about 3 and about 8, as measured by the FTC or ISO method;

cigarette smoke having a pH of greater than about 6; and

a filler having a sugar to nicotine ratio of greater than about 3.5.

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135. A method of making a plant or plant cell culture having an increase in the amount or relative amount of a first alkaloid comprising:

increasing the expression of an first enzyme selected from the group consisting of quinolate phosphoribosyl transferase (QPT) and putrescine N-methyltransferase (PMT) by introducing a nucleic acid encoding QPT or PMT into a plant cell;

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altering, by increasing or decreasing, the expression of at least one additional enzyme involved in a second alkaloid biosynthesis or metabolism of said second alkaloid or its precursors by introducing a nucleic acid into a second plant cell; and

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producing plants or plant cell cultures comprising said first cell and said second cell, wherein the amount of said first alkaloid or the relative amount of said first alkaloid compared to said second alkaloid is greater in said plants or plant cell culture than that in a plant or plant cell culture derived from unmodified plant cells or plant cells in which only the expression of said first enzyme is increased.

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136. The method of claim 135, wherein said first alkaloid and said second alkaloid are selected from the group consisting of nicotine cotinine, nornicotine, myosmine, nicotyrine, anabasine, anatabine, nicotine-n-oxide, N-methyl anatabine, N-methyl anabasine, pseudoxynicotine, 2,3 dipyridyl.

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137. The method of claim 135, wherein said additional enzyme or enzymes are selected from the group consisting of arginine decarboxylase (ADC), methylputrescine oxidase (MPO), NADH dehydrogenase, ornithine decarboxylase (ODC), phosphoribosylanthranilate isomerase (PRAI), putrescine N-methyltransferase (PMT), quinolate phosphoribosyl transferase (QPT), and S-adenosyl-methionine synthetase (SAMS).

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138. The method of claim 135, wherein said first alkaloid in nicotine, said first enzyme is PMT, said additional enzyme is decreased QPT, and said second alkaloid is anabasine.

5 139. The method according to claim 135, wherein said first alkaloid in nicotine, said first enzyme is PMT, said additional enzyme is decreased QPT, and said second alkaloid is anabasine.

10 140. The method according to claim 135, wherein said first alkaloid in nicotine, said first enzyme is PMT, said additional enzyme is decreased QPT, and said second alkaloid is nornicotine.

141. The tobacco plant according to claim 138, 139, or 140.

15 142. The tobacco according to claim 141, and containing reduced TSNAs compared to a unmodified tobacco progenitor plant

143. A cigarette containing the tobacco of claim 142.

20 144. A cigarette comprising:  
an increased-nicotine transgenic plant or plant portion of a species of the genus *Nicotiana*, wherein the production of nicotine in said plant or plant portion is up-regulated as a result of introduction of a synthetic or recombinant nucleic acid into said plant or plant portion or a progenitor plant; and

25 a tar-to-nicotine yield ratio of between about 3 and about 8, as measured by the FTC or ISO method.

30 145. The cigarette according to claim 144, wherein said increased-nicotine transgenic plant or plant portion exhibits increased nicotine as compared to a non-transformed parent plant or plant portion from which said transgenic plant or plant portion is produced.

146. The cigarette according to claim 144, wherein said increased-nicotine transgenic plant or plant portion, as compared to a non-transformed control plant or plant portion, contains and expresses a heterologous DNA encoding at least a segment of an enzyme required for the biosynthesis of nicotine in tobacco, said transgenic plant or plant portion exhibiting increased levels of said enzyme as compared to a non-transformed control plant or plant portion and increased-nicotine content as compared to a non-transformed control plant or plant portion.

147. The cigarette according to claim 146, wherein said enzyme is selected from a group consisting of arginine decarboxylase (ADC), methylputrescine oxidase (MPO), ornithine decarboxylase (ODC), putrescine N-methyltransferase (PMTase), quinolate phosphoribosyl transferase (QPRTase), S-adenosylmethionine synthetase (SAMS), NADH dehydrogenase, and phosphoribosyl anthranilate isomerase (PRAI).

148. A method of making expanded tobacco comprising:  
providing deproteinized tobacco;  
expanding said tobacco; and  
producing a cigarette comprising said tobacco.